

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**

providing a plurality of oligonucleotide probe sets, each set characterized by (a) a first oligonucleotide probe, having an oligonucleotide target-specific portion and an oligonucleotide addressable array-specific portion, wherein the oligonucleotide addressable array-specific portion is comprised of an oligonucleotide sequence that is [separate and] distinct from the oligonucleotide sequence of the target-specific portion, and (b) a second oligonucleotide probe, having an oligonucleotide target-specific portion and a detectable reporter label, wherein the oligonucleotide probes in a particular set are suitable for ligation together when hybridized adjacent to one another on a corresponding target nucleotide sequence, but have a mismatch which interferes with such ligation when hybridized to any other nucleotide sequence present in the sample;

providing a ligase,

blending the sample, the plurality of oligonucleotide probe sets, and the ligase to form a mixture;

subjecting the mixture to one or more ligase detection reaction cycles comprising a denaturation treatment, wherein any hybridized oligonucleotides are separated from the target nucleotide sequences, and a hybridization treatment, wherein the oligonucleotide probe sets hybridize at adjacent positions in a base-specific manner to their respective target nucleotide sequences, if present in the sample, and ligate to one another to form a ligated product sequence containing (a) the addressable array-specific portion, (b) the target-specific portions connected together, and (c) the detectable reporter label, and, wherein the oligonucleotide probe sets may hybridize to nucleotide sequences in the sample other than their respective target nucleotide sequences but do not ligate together due to a presence of one or more mismatches and individually separate during the denaturation treatment;

providing a solid support with different capture oligonucleotides immobilized at particular sites, wherein the capture oligonucleotides have nucleotide sequences complementary to the addressable array-specific portions;

contacting the mixture, after said subjecting, with the solid support under conditions effective to hybridize the addressable array-specific portions to the capture oligonucleotides in a base-specific manner, thereby capturing the addressable array-specific portions on the solid support at the site with the complementary capture oligonucleotide; and

EE  
detecting the reporter labels of ~~ligated~~ product sequences captured to the solid support at particular sites, thereby indicating the presence of one or more target nucleotide sequences in the sample.

---

77. (Amended) A method according to claim 1, wherein the sample potentially contains unknown amounts of one or more of a plurality of target nucleotide sequences with a plurality of sequence differences, said method further comprising:  
providing a known amount of one or more marker target nucleotide sequences;

EE  
providing a plurality of marker-specific oligonucleotide probe sets, each set characterized by (a) a first oligonucleotide probe, having an oligonucleotide target-specific portion complementary to the marker target nucleotide sequence and an addressable array-specific portion complementary to capture oligonucleotides on the solid support, and (b) a second oligonucleotide probe, having an oligonucleotide target-specific portion complementary to the marker target nucleotide sequence and a detectable reporter label, wherein the oligonucleotide probes in a particular marker-specific oligonucleotide set are suitable for ligation together when hybridized adjacent to one another on a corresponding marker target nucleotide sequence, but, when hybridized to any other nucleotide sequence present in the sample or added marker sequences, there is a mismatch which interferes with such ligation, wherein said blending comprises blending the sample, the marker target nucleotide sequences, the plurality of oligonucleotide probe sets, the plurality of marker-specific oligonucleotide probe sets, and the ligase to form a mixture;

detecting the reporter labels of the ligated marker-specific oligonucleotide sets captured on the solid support at particular sites, thereby indicating the presence of one or more marker target nucleotide sequences in the sample; and

quantifying the amount of target nucleotide sequences in the sample by comparing the amount of captured ligated product generated from the known amount of marker target nucleotide sequences with the amount of captured other ligated product.

---

EE  
13. (Amended) A method according to claim 1, wherein the target-specific portions of the oligonucleotide probe sets are configured to be successfully ligated in the presence of their target sequences under a single set of ligase detection reaction [have

23 substantially the same melting temperature so that they hybridize to target nucleotide sequences under similar hybridization] conditions.

---

24 18. (Amended) A method according to claim 1, wherein multiple allele differences consisting of insertions, deletions, microsatellite repeats, translocations, or other DNA rearrangements at one or more nucleotide positions which require overlapping oligonucleotide probe sets in a single target nucleotide sequence or multiple allele differences consisting of insertions, deletions, microsatellite repeats, translocations, or other DNA rearrangements at one or more nucleotide positions which require overlapping oligonucleotide probe sets in multiple target nucleotide sequences are distinguished, the oligonucleotide probe sets forming a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets designed for distinguishing multiple allele differences selected from the group consisting of insertions, deletions, microsatellite repeats, translocations, and other DNA rearrangements at one or more nucleotide positions which require overlapping oligonucleotide probe sets, wherein, in the oligonucleotide probe sets of each group, the second oligonucleotide probes have a common oligonucleotide target-specific portion and the first oligonucleotide probes have differing oligonucleotide target-specific portions which hybridize to a given allele in a base-specific manner, wherein, in said detecting, the labels of ligated product sequences of each group, captured on the solid support at different sites, are detected, thereby indicating a presence, in the sample, of one or more allele differences selected from the group consisting of insertions, deletions, microsatellite repeats, translocations, and other DNA rearrangements in one or more target nucleotide sequences.

---

25 22. (Amended) A method according to claim 20, wherein a low abundance of multiple allele differences at multiple adjacent nucleotide positions, or at nucleotide positions which require overlapping oligonucleotide probe sets, in a single target nucleotide sequence, in the presence of an excess of normal sequence, or a low abundance of multiple allele differences at multiple nucleotide positions which require overlapping oligonucleotide probe sets in multiple target nucleotide sequences, in the presence of an excess of normal sequence, are quantified in a sample, said method further comprising:

providing a known amount of one or more marker target nucleotide sequences;

providing a plurality of marker-specific oligonucleotide probe sets, each set characterized by (a) a first oligonucleotide probe having an oligonucleotide target-specific portion complementary to the marker target nucleotide sequence and an addressable array-specific portion, and (b) a second oligonucleotide probe, having an oligonucleotide target-specific portion complementary to the marker target nucleotide sequence and a detectable reporter label, wherein the oligonucleotide probes in a particular marker-specific oligonucleotide set are suitable for ligation together when hybridized adjacent to one another on a corresponding marker target nucleotide sequence, but, when hybridized to any other nucleotide sequence present in the sample or added marker sequences, have a mismatch which interferes with such ligation;

EF  
COPY

providing a plurality of oligonucleotide probe groups, each group comprised of one or more oligonucleotide probe sets or marker-specific oligonucleotide probe sets designed for distinguishing multiple allele differences at a single nucleotide position, including marker nucleotide sequences, wherein one or more sets within a group share a common second oligonucleotide probe and the first oligonucleotide probes have different target-specific probe portions which hybridize to a given allele or a marker nucleotide sequence excluding the normal allele, in a base-specific manner, wherein said blending comprises blending the sample, the marker target nucleotide sequences, the plurality of oligonucleotide probe sets, the plurality of marker-specific oligonucleotide probe sets, and the ligase to form a mixture;

detecting the reporter labels of the ligated marker-specific oligonucleotide sets captured on the solid support at particular sites, thereby indicating the presence of one or more marker target nucleotide sequences in the sample; and

quantifying the amount of target nucleotide sequences in the sample by comparing the amount of captured ligated products generated from the known amount of marker target nucleotide sequences with the amount of other captured ligated product generated from the low abundance unknown sample.

58. (Amended) A method according to claim 57, wherein the genetic disease <sup>candidate w/ a known</sup> has a known nucleotide sequence and is selected from the group consisting of 21

EC

hydroxylase deficiency, cystic fibrosis, Fragile X Syndrome, Turner Syndrome, Duchenne Muscular Dystrophy, Down Syndrome, heart disease, single gene diseases, HLA typing, phenylketonuria, sickle cell anemia, Tay-Sachs Syndrome, thalassemia, Klinefelter's Syndrome, Huntington's Disease, autoimmune diseases, lipidosis, obesity defects, hemophilia, inborn errors in metabolism, and diabetes.

59. (Amended) A method according to claim 1, wherein said method is used to detect cancer having a known nucleotide sequence and involving oncogenes, tumor suppressor genes, or genes involved in DNA amplification, replication, recombination, or repair.

67. (Amended) A method according to claim 1, wherein the solid support is functionalized with olefin, amino, hydroxyl, silanol, aldehyde, keto, halo, acyl halide, or carboxyl groups to permit attachment of the capture oligonucleotide on the solid support.

138. (Amended) A kit for identifying one or more of a plurality of sequences differing by single-base changes, insertions, deletions, or translocations in a plurality of target nucleotide sequences comprising:

a ligase;

a plurality oligonucleotide probe sets, each characterized by (a) a first oligonucleotide probe, having an oligonucleotide target sequence-specific portion and an oligonucleotide addressable array-specific portion, wherein the oligonucleotide addressable array specific portion is comprised of an oligonucleotide sequence that is distinct from the oligonucleotide sequence of the target-specific portion and (b) a second oligonucleotide probe, having an oligonucleotide target sequence-specific portion and detectable reporter label, wherein the oligonucleotide probes in a particular set are suitable for ligation together when hybridized adjacent to one another on a respective target nucleotide sequence, but have a mismatch which interferes with such ligation when hybridized to any other nucleotide sequence, present in the sample; and

a solid support with capture oligonucleotides immobilized at particular sites, wherein the capture oligonucleotides have nucleotide sequences complementary to the addressable array-specific portions.